

A STUDY OF THE EFFECT OF HIGH AND LOW FAT
DIETS ON THE CHOLESTEROL METABOLISM OF
FOUR GENERATIONS OF WHITE RATS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE UNIVERSITY OF MICHIGAN

By

CARLETON RAYMOND TREADWELL

ANN ARBOR, MICHIGAN

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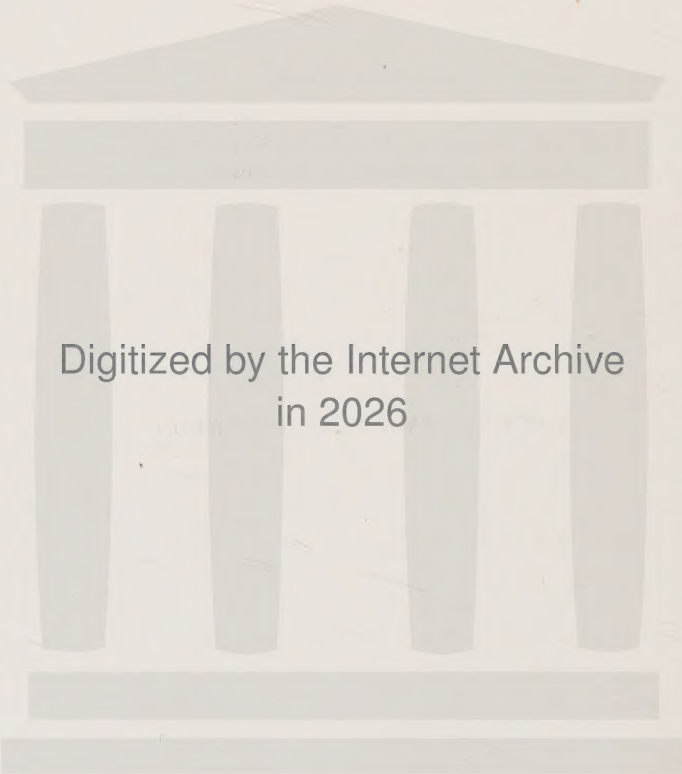
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STEROL METABOLISM IN YOUNG WHITE RATS

IV. THE EFFECT OF HIGH AND LOW FAT DIETS ON THE CHOLESTEROL METABOLISM OF FOUR GENERA- TIONS OF WHITE RATS

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In previous communications from this laboratory (1, 2) it was concluded that the fat content of the diet is intimately associated with the synthesis of cholesterol in the young white rat. This view was based on the observation that the difference between the fecal and dietary sterols was greater on adequate diets containing 28 per cent fat than when the fat content of the diet was only 6 per cent. Although the work presented herein is in one respect a repetition of the previous studies in that the influence of the level of the dietary fat on sterol balance was again determined, the investigation was made more comprehensive in that it was carried out through four generations, all originating from one group of animals. In the case of some of the females separate balances were made during the different phases of the reproductive cycle. The free and total cholesterol of the whole blood and serum as well as the neutral fat, total phospholipids, and sterols in the livers was also determined.

As a result of this present study it was concluded that, although larger amounts of sterols were synthesized when the fat content of the diets was increased from 6 to 28 per cent, this was not reflected in the blood, inasmuch as no differences were observed between the free or total cholesterol contents of the whole blood or serum of the animals on the two types of diets. The neutral fat, phospholipid, and cholesterol contents of the livers were likewise not influenced by the level of fat in the diets.

The following is a description of the experimental procedure em-

ployed. Fifteen young white rats (litter mates, age 50 days) were divided as equally as possible with respect to sex into two groups and placed on the diets described in Table I. One group (A) was given the diet containing 6 per cent fat (Diet 3) and the other group (B) the 28 per cent fat diet (Diet 1). Three generations were obtained from these original two groups, first matings being made when the animals were approximately 100 days old. All descendants of Group A were maintained on the low fat diet, whereas the offspring from Group B were kept on the ration containing the 28 per cent of fat. In view of the smaller caloric

TABLE I
Composition of Diets

All diets were supplemented daily with 2 dry yeast tablets, 2 drops each of a vitamin A and a vitamin B concentrate every other day, and 1 drop of wheat germ oil on alternate days. During gestation and lactation 15 per cent of the starch was replaced in all diets with dried brewers' yeast that had been thoroughly extracted with ethyl ether. The intake of vitamins A, D, and E was 3.5 times as great during these two periods as in the others.

	Diet 1	Diet 2	Diet 3	Diet 4
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Casein.....	30.5	18	27	18
Salt mixture*.....	4.0	4	4	4
Agar.....	4.0	4	4	4
Starch.....	33.5	46	59	68
Mazola.....	28.0	28	6	6

* Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **37**, 572 (1919).

content it was anticipated that the food intake of the rats on Diet 3 would be greater than that of the animals on the high fat Diet 1. For this reason the protein content of Diet 3 was made lower than that of Diet 1 in order that the protein intakes of the rats on both diets should be approximately the same. A subsequent study of the food intakes showed this to be the case. Some of the animals in the third and fourth generations were placed on Diets 2 and 4 which differed from the other two in that the protein contents were lowered to 18 per cent. This change was made in order to ascertain whether the high protein content of Diet 1 might have influenced the cholesterol values. It was believed that all four diets would be adequate for growth, gestation, and lactation. Preliminary experiments, however, showed that, although good growth

and gestation could be secured, the mothers did not suckle their young. Out of approximately 150 young born to mothers on these diets only two were successfully weaned, the rest dying of starvation within 4 or 5 days after birth. However, when 10 gm. of fresh beef liver were included in the daily diet of the mothers, healthy young were obtained at weaning time. This liver was analyzed for total lipid, cholesterol, and protein ($N \times 6.25$) and these were taken into account in calculation of the sterol balances and food intakes. The sterol content of the dietary fat, dietary accessories, feces, and remaining tissues was determined gravimetrically by the digitonin method (3). Samples of whole blood were taken from the tail, whereas blood required for serum analyses was obtained from the heart during light chloroform anesthesia. In both cases the blood was taken after an 18 hour fast. The free and total cholesterol was determined photometrically according to the Schoenheimer and Sperry procedure (4). The rats were kept in individual cages, food intake and changes in body weight were recorded, and collection of feces made. In the case of the males and some of the females the feces were collected as a single sample. Samples during pregestational, gestational, lactational, and postlactational were obtained from the rest of the females. The various phases of the estrual cycle were differentiated from each other by means of the vaginal smear method (5). The method of treatment for the feces, dietary constituents, and remaining tissues prior to sterol analysis has already been described elsewhere (1). The procedure for obtaining the liver lipids has also been previously described (6). The dry total lipids of the liver were redissolved in petroleum ether (b.p. 30–60°) and diluted to a definite volume with that solvent. The aliquots for free and total cholesterol were transferred to centrifuge tubes and the solvent removed by immersing the containers in a water bath. The residue was dissolved in a mixture of anhydrous acetone and absolute alcohol (1:1) and the analysis was completed by the Schoenheimer and Sperry procedure. Aliquots for phospholipids were evaporated to dryness and oxidized with 10 N sulfuric acid and 30 per cent hydrogen peroxide (superoxol). The phosphorus was then determined colorimetrically by the Fiske and Subbarow method (7). Neutral fat was calculated from the difference between the total lipids and the sum of the phospholipids and cholesterol.

Most of the data are summarized in Tables II to V. The

TABLE II

Distribution of Cholesterol in Whole Blood and Serum of Rats Fed High and Low Fat Diets

All values are expressed as mg. per 100 cc. The figures in parentheses show the ranges of the values for the individual rats. The first number in the first column refers to the generation and the second one to the number of the diet.

Generation and diet No.	No. of animals	Cholesterol					
		Whole blood			Serum		
		Total	Free	Ester	Total	Free	Ester
1-1	5	103 (90-114)	93 (85-99)	10 (3-18)	61 (47-79)	20 (13-23)	41 (24-60)
2-1	10	99 (89-116)	85 (77-101)	14 (9-20)	51 (41-59)	11 (8-14)	40 (31-45)
3-1	6	102 (84-121)	84 (70-100)	18 (14-24)	67 (53-83)	21 (17-26)	47 (31-59)
4-1	4	100 (94-110)	81 (78-84)	19 (15-28)	107 (82-127)	22 (14-24)	85 (31-93)
3-2	5	107 (94-122)	85 (80-90)	22 (13-29)	71 (47-100)	18 (11-22)	53 (36-78)
4-2	5	100 (85-111)	82 (70-91)	19 (14-27)	84 (65-105)	22 (12-38)	62 (40-86)
Average		102	85	18	72	19	53
1-3	5	114 (96-127)	96 (90-99)	19 (7-29)	66 (54-76)	17 (14-20)	50 (40-57)
2-3	10	102 (95-107)	85 (79-89)	17 (13-21)	68 (50-87)	16 (13-21)	52 (38-69)
3-3	5	107 (91-113)	90 (77-107)	16 (11-19)	67 (32-87)	22 (10-24)	46 (22-63)
4-3	4	119 (111-125)	103 (97-107)	16 (13-19)	57 (52-68)	17 (15-21)	41 (32-52)
3-4	5	105 (96-109)	85 (75-93)	20 (14-24)	66 (57-75)	21 (16-33)	45 (23-55)
4-4	3	110 (100-122)	86 (74-100)	20 (16-23)	57 (47-65)	17 (11-22)	40 (26-47)
Average		108	88	19	65	19	46
Average, all diets		105	87	18	68	18	49

records on growth have not been included, since no significant differences were observed in this respect in the animals on the different diets.

TABLE III

Distribution of Lipids in Livers of Rats Fed High and Low Fat Diets

All values are expressed as per cent. The figures in parentheses show the ranges of the values for the individual rats. The first number in the first column refers to the generation and the second one to the number of the diet.

Generation and diet No.	No. of animals	Total lipids	Phospho-lipids	Neutral fat	Cholesterol		
					Total	Free	Ester
1-1	5	5.2 (3.8-6.4)	3.1 (2.6-4.1)	2.0 (1.0-3.1)	0.19 (0.16-0.20)	0.14 (0.11-0.18)	0.10 (0.06-0.11)
2-1	10	7.0 (5.4-8.9)	4.0 (3.2-4.5)	2.7 (1.7-4.9)	0.30 (0.19-0.37)	0.22 (0.16-0.28)	0.13 (0.06-0.28)
3-1	6	7.2 (5.3-6.7)	3.9 (2.8-4.4)	2.9 (1.9-4.0)	0.36 (0.27-0.42)	0.23 (0.19-0.30)	0.19 (0.14-0.23)
4-1	4	7.2 (6.5-8.7)	4.3 (3.7-4.9)	2.5 (1.9-3.4)	0.37 (0.35-0.40)	0.27 (0.23-0.31)	0.15 (0.12-0.16)
3-2	5	8.0 (6.3-9.3)	4.5 (3.5-5.0)	2.9 (2.2-3.7)	0.47 (0.41-0.54)	0.27 (0.25-0.29)	0.33 (0.25-0.45)
4-2	5	7.6 (6.9-9.5)	4.5 (3.8-4.8)	2.7 (2.0-3.1)	0.45 (0.38-0.51)	0.26 (0.25-0.28)	0.33 (0.22-0.46)
Average.		7.1	4.2	2.7	0.40	0.24	0.17
1-3	6	6.4 (5.4-7.8)	3.6 (2.7-4.6)	2.5 (1.7-4.2)	0.24 (0.21-0.29)	0.17 (0.14-0.20)	0.12 (0.08-0.21)
2-3	8	6.9 (5.2-8.5)	3.8 (2.9-4.5)	2.8 (1.1-3.7)	0.26 (0.16-0.33)	0.18 (0.13-0.25)	0.13 (0.05-0.27)
3-3	6	6.0 (5.4-6.5)	3.6 (3.0-4.0)	2.2 (1.7-3.0)	0.29 (0.25-0.36)	0.21 (0.17-0.23)	0.13 (0.09-0.23)
4-3	3	5.0 (4.6-5.5)	3.0 (2.9-3.2)	1.5 (1.2-1.9)	0.35 (0.34-0.36)	0.29 (0.26-0.32)	0.11 (0.06-0.15)
3-4	5	7.3 (5.8-9.4)	3.5 (3.2-3.8)	3.1 (2.3-4.2)	0.29 (0.23-0.34)	0.20 (0.16-0.25)	0.14 (0.12-0.17)
4-4	4	4.9 (4.6-5.5)	2.7 (2.5-2.9)	2.0 (1.6-2.6)	0.32 (0.28-0.35)	0.28 (0.22-0.31)	0.08 (0.05-0.11)
Average.		6.3	3.5	2.5	0.28	0.22	0.12
Average, all diets ..		6.7	3.9	2.6	0.35	0.23	0.15

Table II summarizes the data on the whole blood and serum. It is clear from these data that under the conditions obtaining in this study neither the fat nor the protein content of the diets

influenced the fasting level of the different cholesterol fractions of the whole blood or serum. These data become all the more

TABLE IV

Sterol Balances of Rats Fed High and Low Fat Diets

These balances, which are all negative, represent the differences between the dietary and fecal sterols and are expressed as mg. per day. The figures in parentheses show the ranges of the values for the individual rats. The first number in the first column refers to the generation and the second one to the number of the diet.

Generation and diet No.	Males			Females		
	No. of animals	Days on diet	Daily balance	No. of animals	Days on diet	Daily balance
1-1	2	134	9.5 (9.1- 9.8)	1	134	1.7
2-1	6	144	11.6 (8.8-19.6)	3	122	7.4 (4.7-10.5)
3-1	3	199	10.6 (7.9-13.5)	1	147	7.1
4-1	2	126	9.5 (8.2-10.7)	2	126	5.0 (4.4- 5.6)
3-2	2	119	11.7 (9.0-14.0)	1	171	11.0
4-2	3	143	9.2 (8.2-10.5)	2	123	10.3 (10.0-10.5)
1-3	3	134	7.8 (6.4- 8.7)			
2-3	3	121	5.4 (5.1- 5.8)	6	175	4.4 (2.4- 6.5)
3-3	4	158	5.3 (4.1- 6.0)			
4-3	3	99	5.5 (4.9- 5.9)			
3-4	3	93	4.2 (2.6- 4.8)	2	93	3.9 (3.2- 4.6)
4-4	3	120	4.8 (3.7- 6.1)	1	99	5.2
Average, high fat diets ..			10.5			8.2
“ low “ “			5.2			4.4

significant when it is considered that the experiments were carried out through four generations. No significant differences were observed between the sexes and hence no differentiation was made in this respect in summarizing these data.

The data on the livers (Table III), as in the case of the blood, showed no differences with regard to sex and hence the animals were merely grouped with respect to diet. It is at once clear from Table III that the increase in the dietary fat from 6 to 28 per cent did not significantly influence the total lipid content or any of the liver lipid fractions recorded in the table, even though this considerable difference in fat intake was maintained throughout the four generations.

In spite of this constancy in the cholesterol content of the blood and livers an examination of Tables IV and V shows that the average daily negative sterol balances of the rats on the high fat

TABLE V

Sterol Balances during Different Phases of Reproductive Cycles of Rats Fed High and Low Fat Diets

These balances which represent the differences between the dietary and fecal sterols are expressed as mg. per day. The values in parentheses show the ranges of the values for the individual periods.

Phase	High fat Diets 1 and 2		Low fat Diets 3 and 4	
	No. of periods	Balance	No. of periods	Balance
Pregestation.....	5	-5.1 (4.0- 6.6)	5	-2.4 (0.32- 4.8)
Gestation.....	7	-6.2 (4.1-10.8)	6	-4.3 (3.3 - 5.1)
Lactation.....	8	+15.4 (7.4-21.0)	5	+11.5 (9.2 -15.2)
Postlactation....	8	-4.2 (3.3- 7.0)	6	-4.2 (3.5 - 4.9)

diets were greater than those of the animals on the low fat régime. Table IV summarizes the data on all of the males and females in which the feces were collected during the whole experimental period. It is clear from these data that this difference in the sterol content referred to above was maintained throughout the four generations of males. The same general trend was observed for the females. There are, however, three exceptions to this, one occurring in the female in the first generation and two in the fourth generation. Table V gives the data on the balances of some of the females during the different phases of the reproductive cycle. It is clear that even during gestation when additional cholesterol is required for the growing embryos the balances still remain negative. On the other hand distinctly positive balances became evident during lactation. This might have been anticipated because

of the probability of a considerable loss of cholesterol from the body during this time by way of the milk. It may, however, not be just to compare the data obtained during this phase with those in the other periods, since it was necessary to include liver in the diets of the mothers in order to have them nurse their young. Thus considerable amounts of the readily absorbable sterol, cholesterol, were administered to these mothers. For this reason a definite conclusion with respect to the balances during lactation must be withheld.

SUMMARY

1. The differences between the fecal and dietary sterols of four generations of rats were greater on an adequate diet containing 28 per cent fat than when the fat content of the diet was 6 per cent.

2. The free and total cholesterol content of the fasting whole blood and serum of the four successive generations was not influenced by the level of fat in the diet.

3. The neutral fat, phospholipid, and cholesterol contents of the livers of these rats were likewise not influenced significantly by the increase in dietary fat.

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